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REVIEW

The spectrum of use of rituximab in chronic lymphocytic leukemia

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Abstract: The monoclonal chimeric anti-CD20 antibody, rituximab, has considerably improved therapeutic outcome in B-cell chronic lymphocytic leukemia. Rituximab has limited clinical activity when used as a single agent. The combination of the monoclonal antibody with fludarabine-based regimens clearly demonstrated, in Phase II and randomized trials, an increase in clinical efficacy in previously untreated and pretreated patients. Furthermore the addition of rituximab enabled the eradication of minimal residual disease, which is correlated with the prognosis in a high proportion of patients. Although the combination of rituximab with fludarabine-based regimens increased myelosuppression and immunosuppression, incidence of infections did not increase. The benefit of adding rituximab to other purine analogs or other chemotherapeutic combination regimens has also been explored. Moreover there could be a role for achieving better quality of responses with the combination of different monoclonal antibodies, considering that they target different antigens and exert different mechanism of action. Although the role of rituximab as maintenance therapy in low grade non-Hodgkin's lymphomas has been determined, the benefit and optimal schedule in chronic lymphocytic leukemia are still under investigation. This review brings together knowledge of the pharmacokinetics, mechanism of action and clinical use of rituximab in chronic lymphocytic leukemia.

Keywords: rituximab, B-cell chronic lymphocytic leukemia, first-line treatment, refractory/relapsed

Introduction

B-cell chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries, with an annual incidence of 3 to 5 cases per 100,000.[1] The disease is rare in people younger than 50 years, but incidence rises fairly rapidly above this age. The median age at diagnosis, according to Surveillance, Epidemiology and End Results (SEER), is 70 years for men and 74 years for women.[1]

Overall survival (OS) rate in CLL after diagnosis ranges from less than 2 to more than 15 years, with a median of 9 years, because the clinical course is extremely variable. A third of patients never need treatment and have long survival, in another third an initial indolent phase is followed by disease progression, and the remaining third exhibit aggressive disease at onset and need immediate treatment.[2]

Although clinical stagings proposed in the early 1980s by Binet et al[3] and Rai et al[4] are still widely used as prognostic factors in CLL, these systems do not enable identification of patients in early stages who are likely to progress and those in whom the disease will remain stable for many years.

In recent years, several biological markers – mutation status of the immunoglobulin heavy chain variable chain (IgVH), expression of ZAP-70, expression of CD38,

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chromosome abnormalities identified by fluorescence in situ hybridization (FISH) analysis, and P53 mutations – have been identified as important prognostic factors.[5–10] These factors, as well as other historical serological parameters such as beta2-microglobulin,[11] proved to be useful in predicting which patients would develop progressive disease. Furthermore they may provide prognosis assessment for response to treatment, response duration and survival.

In routine clinical practice, therapy should not be initiated in patients who have asymptomatic CLL, including those with Rai stage 0 or Binet stage A, until disease progression or unless disease-related symptoms are evident. The observation that early treatment in asymptomatic patients does not prolong survival is supported by a meta-analysis.[12] In seven trials including 2048 early-stage patients randomly allocated to immediate or deferred treatment with chlorambucil (with or without prednisolone) no benefit for either treatment group was observed.

However the potential benefit of an early intervention therapy according to prognostic factors and novel agents, including monoclonal antibodies, requires further study. Current trials are ongoing in Europe and in the United States (US) to re-address this issue using more efficient treatments and considering patients with high-risk features.

Widely accepted guidelines for the initiation of chemotherapy in CLL have been proposed by the National Cancer Institute Sponsored Working Group[13] and have been recently reviewed by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL).[14] According to these guidelines the criteria for initiation of therapy may not be identical for routine clinical practice and for clinical trials.

Despite improvement in therapy, CLL remains incurable with standard therapy. Patients inevitably relapse, becoming increasingly refractory to treatment and often acquiring high-risk chromosomal abnormalities. In the past two decades the introduction of purine analogs, most notably fludarabine, has had an important effect on CLL management.[15] Fludarabine has demonstrated greater efficacy than chlorambucil or CAP (cyclophosphamide, doxorubicin and prednisone) in obtaining higher response rates, complete remission (CR) rates and progression-free survival (PFS).[16–18] The advantages were greater when purine nucleoside analogs were combined with cyclophosphamide and/or mitoxantrone.[19–21]

However studies with purine analogs-based chemotherapy failed to show an advantage in terms of OS.[22] The same observation can be applied when considering results obtained with other chemotherapeutic agents administered alone or in combination treatments.

A recent advance in the treatment of CLL is the development of monoclonal antibodies directed against specific proteins expressed by CLL B-cells. Both alemtuzumab and rituximab, the two antibodies with the most clinical value in CLL, were shown to be active when used in monotherapy.[23,24] The preclinical evidence of the synergistic effect between monoclonal antibodies and chemotherapeutic agents[25,26] and the need for new association treatments, based on drugs with different mechanisms of actions possibly without adding major toxicities, prompted the investigation of immunochemotherapeutic regimens. Treatment strategies including monoclonal antibodies not only improved response rates, but also achieved better quality of responses, with minimal residual disease (MRD) being eradicated in a significant proportion of patients.[27–29]

The achievement of MRD, as determined by flow-cytometry or polymerase chain reaction (PCR)-based amplification of the IgVH rearrangement, is correlated with an improvement in time to retreatment and PFS.[27–31] In 2005, Moreton et al first correlated the eradication of MRD with an improvement of OS after alemtuzumab monotherapy treatment in relapsed CLL patients.[32] However, even if immunochemotherapy has eradicated MRD, patients will eventually relapse, as published trials have failed to demonstrate a plateau of PFS and OS curves. Although eradication of MRD may improve prognosis, prospective clinical trials are needed to define whether additional treatment administered to eradicate MRD provides significant benefit to clinical outcome.

To address this issue, MRD negativity has been adopted as a recommended trial endpoint in the recently updated diagnostic and treatment guidelines of the IWCLL working group.[14]

Allogeneic stem cell transplantation offers the possibility of a definite cure, but despite recent developments, such as reduced intensity conditioning and better supportive care, it is still associated with significant morbidity and mortality. However, in patients carrying del17p, requiring treatment, an allogeneic transplant should be considered as a therapeutic option that may induce long-lasting remissions.[33]

The current review brings together knowledge of the pharmacokinetics, mechanisms of action, and clinical use of rituximab in CLL.

Mechanism of action, pharmacokinetics of rituximab

Rituximab is a chimeric human/mouse Ig1-kappa antibody that targets the CD20 antigen. The chimeric structure of rituximab incorporates murine variable regions and human

constant kappa and Fc regions to diminish the development of anti-mouse immunoglobulin antibody side effects and possibly resistance.[34]

CD20, a cell-surface glycoprotein, is a calcium channel that interacts with the B-cell immunoglobulin receptor complex. CD20 is tightly restricted to the B-cell lineage and is expressed on the cell surface of more than 95% of normal and malignant B-cells, excluding stem cells and plasma cells.[35] However there are several differences in CD20 expression between B-cell malignancies. In contrast to B-cell lymphomas, which uniformly express CD20 strongly, relatively low levels of CD20 are typically expressed in CLL.[36]

Rituximab exerts its anticancer effects through more than one mechanism of action. The predominant mechanisms of rituximab-induced cell death are proposed to be the result of antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and apoptosis.[37–41]

The relative importance of these mechanisms may differ between CLL and B-cell non-Hodgkin's lymphomas (NHL).

ADCC seems to be the predominant mechanism for the clearance of neoplastic cells in lymphomas, and Fc-γ receptors are critical for the in vivo actions of rituximab. The difference in response rates among lymphoma patients according to Fc-γ RIIIa polymorphisms supports the importance of ADCC in the in vivo actions of rituximab.[42] In contrast, in CLL Fc-γ RIIIa polymorphisms are not predictive for response.[43] In vitro studies with rituximab have shown CDC to be more rapid and effective at inducing cell death than ADCC or apoptosis,[40,41] particularly for blood-borne diseases, such as the leukemic-phase of B-cell NHL, including CLL, in which access to complement may be enhanced. Complement activation may be important, as increased expression of complement inhibitors CD55 and CD59 resulted in resistance to rituximab in B-NHL cell lines and CLL cells.[44,45] However caspase-3 activation and induction of apoptosis, using a pathway similar to that of fludarabine and other chemotherapeutic agents, appear to play a more important role in CLL than in B-cell NHL.[44,46,47]

In addition to the efficacy of rituximab in inducing a proapoptotic signal via the cell surface target structure, several studies have pointed out the activity of rituximab in promoting cellular responses against tumors. Selenko et al demonstrated in vitro that the monoclonal anti-CD20 antibody promotes uptake and cross-presentation of lymphoma cell-derived peptides by antigen-presenting dendritic cells inducing maturation of dendritic cells, and allows the generation of specific cytotoxic T-cells that may have a long-lasting protective effect.[48,49] Although this mechanism remains to be proved in vivo, the evidence of tardive and prolonged responses to rituximab treatment suggests that there is the potential for long-lasting antitumor protection.

Rituximab as a single agent

Single-dose Phase I trials showed the antibody to be well tolerated, with a half-life of 4 days.[50] Multiple-dose schedules were developed and the Phase II trials explored a four-dose schedule.[51,52] In the US Phase II trial 166 patients with relapsed low-grade lymphomas received rituximab at a dose of 375 mg/m^2 weekly for four consecutive weeks.[52] The subgroup of 33 patients with small lymphocytic lymphoma (SLL), the tissue counterpart of CLL, achieved a significantly lower response rate than those with follicular lymphoma (12% vs 58%; $P < 0.01$). Subsequent studies in relapsed CLL with rituximab administered at a dose of 375 mg/m^2 weekly for 4 weeks also showed a lower response rate than usually seen in NHL, with almost all responses being categorized as partial remissions (Table 1).[51,53–60]

Two studies explored the efficacy of rituximab in first-line treatment.[59,60] Hainsworth et al achieved an initial overall response rate (ORR) of 51% (4% CR) after initial standard rituximab regimen, increasing to 70% after maintenance therapy in CLL/SLL patients.[59] A high ORR (90%, 9% CR), was also reported by Thomas et al in 21 CLL patients with Rai stage 0, I, II disease and elevated beta2-microglobulin level without other indications of therapy.[60] Although rituximab used in first-line treatment was shown to be more effective than in the setting of relapsed/refractory patients, the drug in CLL/SLL is still less active than in follicular lymphomas. Furthermore, better quality of responses and more prolonged PFS can be obtained with fludarabine in monotherapy, albeit with more substantial toxicity.[61]

Several explanations have been considered for the reduced responsiveness of CLL/SLL to monoclonal antibody treatment. CD20 expression in CLL/SLL is significantly lower than in other types of NHL, which may affect the degree of antibody binding.[37] However Perz et al could not identify a correlation between CD20 expression and efficacy of rituximab treatment.[62] Another mechanism could be the rapid clearance of the antibody from the circulation due to the high number of circulating cells in CLL, which may give a lower serum concentration. Furthermore, because high levels of soluble CD20 have been demonstrated in plasma of patients with CLL,[63] it is theoretically possible that circulating CD20 may deplete the concentration of rituximab available for binding to the tumor cells.

Table 1 Rituximab as a single agent

Authors	No. evaluable pts	Disease status	Rituximab schedule	Response
Winkler et al[53]	9	Pretreated	375 mg/m^2/w iv for 4 weeks	1 (11%) PR
Nguyen et al[54]	15	Pretreated	375 mg/m^2/w iv for 4 weeks	1 (7%) PR
Foran et al[51]	29	Pretreated	375 mg/m^2/w iv for 4 weeks	4 (14%) PR
Huhn et al[55]	28	Pretreated	375 mg/m^2/w iv for 4 weeks	7 (25%) PR
Itala et al[56]	23	Pretreated	375 mg/m^2/w iv for 4 weeks	8 (35%) PR
O'Brien et al[57]	24	Pretreated	375 mg/m^2 iv wk 1, 500–825 mg/m^2 iv weeks 2,3,4	5 (21%) PR
	7		375 mg/m^2 iv wk 1, 1000–1500 mg/m^2 iv weeks 2,3,4	3 (43%) PR
	8		375 mg/m^2 iv wk 1, 2250 mg/m^2 iv weeks 2,3,4	6 (75%) PR
Byrd et al[58]	29	Pretreated	250 mg/m^2/ tiw iv for 4 weeks	14 (48%) PR
		Untreated	375 mg/m^2/ tiw iv for 4 weeks	1 (4%) CR
Hainsworth et al[59]	43	Untreated	375 mg/m^2/w iv for 4 weeks	22 (51%) OR 2 (4%) CR
Thomas et al[60]	21	Untreated	375 mg/m^2/w iv for 4 weeks	19 (90%) OR 2 (9%) CR

Abbreviations: pts, patients; w, weekly; iv, intravenous; PR, partial response; CR, complete response; OR, overall response; tiw, 3 times a week.

Whatever the reason for the poorer responses, pharmacokinetic studies conducted in relapsed refractory NHL showed markedly lower levels of circulating rituximab in the serum of patients with Working Formulation A disease.[52]

The standard regimen of 375 mg/m^2 weekly for 4 weeks was established for the treatment of NHL on the basis of efficacy and maximum tolerated dose in a dose escalation study; however patients receiving higher dosages of the monoclonal antibody showed no evidence of dose-limiting toxicity.[64] This was the rationale for increasing dosing of rituximab in CLL in order to maintain high antibody concentrations in serum and possibly overcome its rapid clearance by the large number of circulating CLL cells.

Both dose-intense and dose-intensity strategies have been considered in previously treated patients.

O'Brien et al designed a dose escalation study with an initial infusion of 375 mg/m^2 followed by three further escalating doses ranging from 500 mg to 2250 mg/m^2.[57] Results of this study showed that responses were strongly correlated with the dose administered: 21% of responses with the dose of 500 mg/m^2 compared with 75% after the administration of the higher dose of 2250 mg/m^2. Toxicity in the dosage range of 500 mg up to 1500 mg/m^2 was very uncommon, and maximum tolerated dose was 2250 mg/m^2. Infusion-related events were mostly associated with the first infusion of 375 mg/m^2.

In another study of dose intensity, Byrd et al increased the frequency of the standard rituximab dose to 3 times per week. The same regimen was applied in six untreated patients, and an ORR of 83% was obtained.[58]

Even if these studies indicated that increased doses of rituximab are effective, median time to progression was of short duration in both studies (8 and 11 months, respectively). Furthermore these schedules are not applicable in routine medical practice and, therefore, other strategies to increase the efficacy of rituximab have been considered.

Rituximab in combination with purine analogs

Rituximab plus fludarabine

Rituximab enhances the activity of purine analog-based therapies and has been incorporated into immunochemotherapy regimens.[39]

In vitro studies have demonstrated that apoptosis is enhanced in an additive or synergistic manner when rituximab is combined with chemo- or immunotherapy molecules. Cultured CLL and NHL cells treated with 10 μg/mL rituximab lessened the inhibitory concentration needed by various chemotherapeutic agents to induce apoptosis via increased p53 and activated caspase-3, -7, and -8, irrespective of the addition of exogenous complement proteins.[65,66] Rituximab, by downmodulating the expression of the antiapoptotic protein bcl-2, may sensitize leukemia cells to fludarabine-induced apoptosis.[25] Furthermore, fludarabine downmodulates expression of complement resistance proteins CD46, CD56, and CD59 on malignant B-cells and renders them more susceptible to rituximab-induced CDC.[45]

Fludarabine is one of the most commonly used chemotherapeutic agents in CLL, having an independent single-agent activity and no overlapping toxicity with rituximab. As the purine analog synergizes in vitro with rituximab, most studies of immunochemotherapy have focused on regimens of combinations of rituximab with fludarabine or fludarabine.

The Cancer and Leukemia Group B (CALGB) conducted a randomized Phase II clinical trial to determine the ideal administration schedule of rituximab plus fludarabine in untreated patients (CALGB 9712).[67] Patients were

randomized to receive one course every 4 weeks for 6 months of concurrent fludarabine and rituximab followed by four rituximab consolidation courses or sequential treatment of fludarabine alone followed by four infusions of rituximab as consolidation.

The concurrent schedule was shown to be more effective than the sequential in achieving a higher CR rate (47% vs 28%), while the ORR (90% vs 77%) and PFS did not differ significantly between the two groups. Recently updated long-term follow-up of patients enrolled on CALGB 9712 demonstrated extended OS and PFS, with an estimated 17% of responders still in remission after 8 years.[68] The results of this study were retrospectively compared with the outcome of patients treated with fludarabine alone in the CALGB 9011 study.[69] Patients assigned to receive fludarabine and rituximab had a higher incidence of CR and a significantly improved 2-year PFS and OS compared with patients receiving the purine analog alone. It is worth noting that 74% of patients treated with rituximab combined with fludarabine showed a higher incidence of grade 3–4 neutropenia, although no difference in other hematological toxicity or infection development was noted.

The promising results obtained in the CALGB 9712 are in line with those of the Phase II study of the German CLL study group (GCLLSG).[70] Fludarabine at the standard dose of 25 mg/m^2 for four cycles associated with four infusions of rituximab led to an ORR of 87% with a CR rate of 33%. Similar responses were obtained in both untreated or previously treated patients enrolled in the study. This response rate was achieved after the administration of four courses of treatment and compares favorably with those reported with the most effective chemotherapy combinations in which six courses are generally administered,[71–73] thus allowing the administration of a lower total dose of fludarabine.

One of the major risks when combining rituximab, which causes a prolonged depletion of B-cell lymphocytes, with fludarabine, which is responsible for profound and prolonged T-cells depletion, is the development of opportunistic infections. Both these studies showed that the frequency of infections was not increased after the immunochemotherapy treatment. Most of the opportunistic infections observed were of viral origin and this warrants an adequate prophylaxis against herpes virus.

Rituximab plus fludarabine and cyclophosphamide

Phase II trials

The combination of fludarabine, cyclophosphamide and rituximab (FCR) was first evaluated at the MD Anderson Cancer Center in previously treated and untreated CLL patients.[30,74] Fludarabine and cyclophosphamide (FC) were administered at doses of 25 and 250 mg/m^2, respectively, for 3 days. To prevent the risk of tumor lysis syndrome the first dose of rituximab was 375 mg/m^2 increasing to 500 mg/m^2 from the second to the sixth doses.

In the 177 previously treated patients the ORR was 73%, 25% of cases reached a CR and median time to progression of responding patients of 28 months.[74] The results of this study revealed some important issues. Even if the comparability with previously reported Phase II studies is limited, the authors reported higher responses and CR rates and longer PFS with the combination immunochemotherapy compared with those reported for FC alone.

Furthermore previous treatment with FC does not have a detrimental effect on response to FCR, as in this group of patients CR and OR rates obtained were similar to those for the entire group and to those for patients treated with only alkylating agents. Number of responses observed in the fludarabine-refractory group of patients (6% CRs, 58% ORs) was lower, even though the results were comparable with those reached after salvage treatment with alemtuzumab in the same setting of patients.[75,76]

It should be emphasized that all six intended courses of treatment were administered to less than 50% of patients (46%). Dose reduction occurred in 25 cases, myelosuppression being the primary reason for dose reduction. Grade 3–4 neutropenia occurred in 62% of courses, and major infections were observed in 5% of courses (16% of patients).

An impressively high ORR together with a good quality of responses has been observed after FCR in first-line treatment. Keating et al reported an ORR of 95% with 70% of CRs in 224 untreated patients.[30] Notably, of the 207 patients evaluated at the end of treatment with flow-cytometry studies, 67% had less than 1% CD5 and CD19 co-expressing cells. This study underlined the importance of eradicating MRD, as there was a strong correlation between probability of relapse and responses as well as CD5/CD19 determined by flow-cytometry response.

Compared with the historical group of patients treated with FC, a higher incidence of myelosuppression with a grade 3–4 neutropenia was detected in 52% of courses, and in 38% after chemotherapy, alone even though the incidence of major infections was similar in 2.6% of courses.[30] Time to progression and time to treatment failure survival were significantly better with FCR than with FC.

Recently Tam et al reported the update of the MD Anderson Cancer Center experience on 300 patients receiving FCR

as initial therapy.[27] The impressive clinical activity was confirmed by a CR rate of 72% and 42% of CR patients reaching PCR negativity. Six-year OS and failure-free survival were 77% and 51%, respectively, and median time to progression was 80 months. MRD negativity, determined by flow-cytometry and PCR, was correlated with superior time to progression (median 85 vs 49 months) and survival (84% vs 65%) at 6 years. Karyotypic abnormalities other than those involving chromosome 17 had no effect on CR or survival. In addition it should be noted that immunochemotherapy with FCR may overcome the adverse prognostic significance of del11q.[77] The extended follow-up of patients permitted the evaluation of late toxicity during remission.[27] Following completion of therapy, 19% of patients had persistent cytopenia lasting more than 3 months. Recurrent late cytopenia episodes occurred, predominantly during the first year, in 28% of 245 patients; the risk of serious or opportunistic infection was 10% and 4% during the first and second years of remission, respectively.

Phase III trials

The benefit of adding rituximab to FC has also been examined in prospective randomized trials in previously treated or untreated patients.

The REACH trial was designed to directly compare FCR with FC alone in patients with previously treated CLL.[28] The findings of this multicenter randomized trial including 552 patients supported the results reported in Phase II single-institution studies.[74,78] In the group of patients randomized to receive the monoclonal antibody, significant improvement of ORR, CR and PFS was observed. Of note, both Binet stage B and C patients benefited from FCR as did patients with poor prognostic features such as del11q, unmutated IgVH and positive ZAP-70. Overall survival was not significantly improved but follow-up was relatively short and a post-trial crossover to rituximab had occurred. As reported in other studies, patients treated with FCR showed a higher rate on grade 3–4 neutropenia even if there was no increase in overall or severe infections.

The superiority of FCR over FC was also confirmed in the 877 untreated patients enrolled in the randomized GCLLSG (CLL8).[79] FCR induced a higher ORR than FC (95 vs 88%), more CRs (44% vs 22%; $P < 0.001$) and longer median PFS (51.8 months vs 32.8 months). It should be emphasized that this is the first randomized study to demonstrate a superiority in OS (at 37.7 months 84.1% vs 79.0%) between the two treatment arms, even if this superiority was demonstrated only in Binet stages A and B patients.[31]

The importance of eradicating MRD was confirmed by the GCLLSG CLL8 trial, which demonstrated that median PFS depended on the ability to eradicate MRD in the peripheral blood.

Hematological toxicity was higher in the FCR group of patients, even though the mean number of courses delivered in the two groups of patients was similar, 5.2 in the FCR arm versus 4.8 courses in the FC, with a total median cumulative dose of chemotherapy applied per patient. No statistically significant difference between the two treatments was observed.

The results of Phase III trials of rituximab combined with fludarabine alone (FR) or with cyclophosphamide (FCR) are summarized in Table 2.

In a randomized study of untreated patients, FCR was shown to be more effective and to have a better safety profile compared with the same regimen combined with alemtuzumab (FCCam). This study was closed prematurely because of excessive toxicity observed in the FCCam arm.[80]

The efficacy of the FCR combination as well as the possible associated toxicities led to the investigation of two different regimens in which the three drugs were combined at different dosages or were administered sequentially with the aim of reducing toxicity while preserving efficacy.[81,82]

Foon et al tested a reduced version of FCR (FCR-Lite) in untreated patients.[81] Fludarabine was dose reduced to 20 mg/m^2 and cyclophosphamide to 150 mg/m^2 while rituximab was increased to 500 mg/m^2 on days 1 and 14 of a 28-day cycle and was also given as consolidation treatment until relapse. All the 48 assessed patients responded to treatment, 38 patients reaching a CR (79%) and 37 showing a negative flow-cytometry. Similar to other studies with FCR, the high-risk feature of del17p was associated with a poor response. None of the patients who entered CR showed disease progression after a median of 22.3 months. Even if the reported follow-up is rather short to enable the outcome to be compared with other FCR studies, this study demonstrated that a high ORR can be reached with a reduced dose of chemotherapy, clearly showing a major reduction in grade 3–4 neutropenia (13% of cycles).

At the Memorial Sloan-Kettering Cancer Center, fludarabine, cyclophosphamide and rituximab were sequentially administered.[82] Initially patients received fludarabine for 6 cycles at the standard dose of 25 mg/m^2 for 5 days, thereafter cyclophosphamide was given as consolidation treatment for 3 cycles at the dose of 3000 mg/m^2 every 3 weeks followed by consolidation with rituximab infusions once a week for four weeks. The sequential regimen showed significant efficacy, with an ORR of 89% including 61% of

Table 2 Phase III trials of rituximab with fludarabine alone or in combination with cyclophosphamide

Authors	Comp. study	No. evaluable pts	Disease status	Treatment regimen	Clinical response		Follow-up
					CR (%)	OR (%)	
Byrd et al[67]	Yes	104	Untreated	Sequential: F 25 mg/m^2 iv × 5 d 6 cycles, after 2 mo R 375 mg/m^2 iv 4 weekly dose	47	90	All enrolled patients:
				versus			
				Concurrent: F 25 mg/m^2 iv × 5 d 6 cycles, R 375 mg/m^2 iv d 1 and 4 cycle 1, d 1 of cycles 2–6 after 2 mo R 375 mg/m^2 iv 4 weekly doses	28	77	2 y PFS probability 0.67 2 y OS probability 0.93
Schulz et al[70]	No	31	20 untreated	Concurrent: F 25 mg/m^2 iv d 1–5, 29–33, 57–61, 85–89 and R 375 mg/m^2 iv d 57, 85, 113, 151	20	85	Median DoR 75 weeks
			11 relapsed		27	90	
Wierda et al[74]	No	177	Pretreated	FCR: R 375 mg/m^2 iv first cycle, 500 mg/m^2 d 1 cycles 2–6, F 25 mg/m^2 iv and CTX 250 mg/m^2 iv d 2–4 cycle 1, d 1–3 cycles 2–6 every 4 weeks	25	73	Median TTP: 28 mo Estimated median OS: 42 mo
Tam et al[27]	No	300	Untreated	FCR: R 375 mg/m^2 iv first cycle, 500 mg/m^2 iv d 1 cycles 2–6, F 25 mg/m^2 iv and CTX 250 mg/m^2 iv d 2–4 cycle 1, d 1–3 cycles 2–6 every 4 weeks	72	95	Median TTP: 80 mo 6 y OS 77% 6 y FFS 51%
Robak et al[28]	Yes	552	Pretreated	FC: F 25 mg/m^2 iv and CTX 250 mg/m^2 iv d 1–3 every 4 weeks	13	58	Median PFS 20.6 mo, OS 52 mo
				versus			
				FCR: R 375 mg/m^2 iv first cycle, 500 mg/m^2 iv d 1 cycles 2–6, every 4 weeks F 25 mg/m^2 iv and CTX 250 mg/m^2 iv d 1–3 every 4 weeks	24	70	Median PFS 30.6 mo, OS NR
Hallek et al[79]	Yes	817	Untreated	FC: F 25 mg/m^2 iv and CTX 250 mg/m^2 iv d 1–3	22	88	OS rate at 37.7 mo: 79%
				versus			
				FCR: R 375 mg/m^2 iv first cycle, 500 mg/m^2 iv d 1 cycles 2–6, F 25 mg/m^2 iv and CTX 250 mg/m^2 iv d 1–3	44	95	OS rate at 37.7 mo: 84.1%
Lepretre et al[80]	Yes	100	Untreated	FCR: F 40 mg/m^2 oral d 1–3 and CTX 250 mg/m^2 oral d 1–3 plus R 375 mg/m^2 iv d 0 at first cycle and 500 mg/m^2 d 1 all subsequent cycles	78	96	Not evaluable
				versus			
				FCCam: F 40 mg/m^2 oral d 1–3 and CTX 250 mg/m^2 oral d 1–3 plus Cam 30 mg sc d 1–3	58	85	
Foon et al[81]	No	48	Untreated	FCR-Lite: F 20 mg/m^2 iv d 2–4 first cycle, d 1–3 cycles 2–6, CTX 150 mg/m^2 iv d 2–4 first cycle, d 1–3 cycles 2–6	77	100	Median DoR 22,3 mo
				R 375 mg/m^2 iv d 1 cycle 1, 500 mg/m^2 iv d 14 cycle 1, R 500 mg/m^2 iv d 1, 14 cycles 2–6; maintenance R 500 mg/m^2 iv every 3 mo			None of the CR pts relapsed
Lamanna et al[82]	No	36	Untreated	Sequential FCR: F 25 mg/m^2 iv for 5 d every 4 weeks for 6 cycles, after 4–6 weeks CTX 3000 mg/m^2 iv every 3 weeks for 3 doses, after 4 weeks 375 mg/m^2 iv once weekly for 4 doses	61	89	Median DoR 43 mo

Abbreviations: comp., comparative; pts, patients; CR, complete response; OR, overall response; OS, overall survival; PFS, progression-free survival; DoR, duration of response; TTP, time to progression; F, fludarabine; R, rituximab; CTX, cyclophosphamide; Cam, Campath-1H (alemtuzumab); d, days; mo, months; iv, intravenous; sc, subcutaneous; NR, not reached; FCL-Lite, FCR reduced version.

CRs. The majority of the enrolled patients presented a high-risk disease according to stage and prognostic features. The addition of rituximab improved the CR rate and the quality of responses: MRD eradication determined by flow-cytometry or by patient/tumor-specific nucleotide primers PCR was achieved in 56% and 33% of cases, respectively.

Rituximab in combination with other purine analogs

Pentostatin, among purine analogs active in CLL, appears to be less myelosuppressive than fludarabine. The degree of myelosuppression seems to be favorable, even when pentostatin is combined with cyclophosphamide, compared with fludarabine-based combinations.[83] Several studies have investigated the efficacy of the combination pentostatin, cyclophosphamide and rituximab (PCR) either in previously treated or untreated CLL patients (Table 3).

In a cohort of 32 previously treated CLL patients, Lamanna et al reported an ORR of 75%, including 25% of CRs, with a median response duration of 25 months and a median time to treatment failure of 40 months.[84] These authors compared their results with those obtained at the MD Anderson Cancer Center after FCR treatment in the same setting of patients.[74] Even if there are inherent limitations in this comparison, they noted that responses were virtually identical in the two treatment regimens and median survival of all patients was comparable. The frequency of grade 3–4 neutropenia and infectious complications including fever of unknown origin reported after PCR were inferior to those reported after FCR. Moreover, PCR was better tolerated, as a high proportion of patients received all planned therapy at full dose (72% vs 38% with FCR).

The efficacy of PCR, with pentostatin administered at the dose of 2 mg/m^2, has also been documented in 64 untreated patients.[85] In a subsequent study Shanafelt et al reported that PCR can be safely administered to older patients (≥70 years) and those with modestly decreased creatinine clearance.[86] These results appear to contrast with the tolerability of the FCR regimen: in the PCR study older patients were as likely as younger patients to complete the intended 6 cycles and to achieve CR (41 vs 39%) or PR (52 vs 44%) without excess of grade 3–4 toxicity.

To find out whether results obtained with immunochemotherapy regimens in academic centers are reproducible in the community setting, Reynolds et al carried out a Phase III randomized trial to compare FCR and PCR in previously untreated or minimally treated B-cell CLL.[87] The primary endpoint, incidence of grade 3–4 infections, was similar in the two arms. Only 50% of patients in both arms completed therapy, resulting in low OR rates that were not statistically different between the two treatment groups. The reason for not completing therapy could be related to the choice of drug dosages. Pentostatin was administered at the higher dosage of 4 mg/m^2 while in the FCR regimen patients received fludarabine 20 mg/m^2 for 5 days and cyclophosphamide at 600 mg/m^2 in 1 day.

To improve tolerability while preserving efficacy, the higher dosage of pentostatin (4 mg/m^2) has also been used in association with rituximab, without cyclophosphamide, in first-line treatment.[88] This combination led to lower OR (76%) and CR rates (27%) as well as shorter treatment-free survival compared with the PCR regimen. These results support previous findings on the importance of adding cyclophosphamide to purine analogs and rituximab to improve response rates and prolong PFS.

There is only one published study on the combination of rituximab and intravenous cladribine (RC) with or without cyclophosphamide in refractory and relapsed CLL patients. The objective response observed (78%) was in line with the other immunochemotherapy regimens and superior to cladribine used alone or in combination with cyclophosphamide. As observed when combining rituximab with fludarabine, the addition of the monoclonal antibody to cladribine did not confer a higher infectious rate. Even though this is a small series of patients, the authors noted that the response ratio was similar in patients treated with or without cyclophosphamide.[89]

The efficacy on CLL and SLL of cladribine administered subcutaneously with rituximab has been recently reported.[90] Considering that four courses of therapy were administered and that 38% of patients had been previously treated, an impressive 50% CRs (54% untreated; 44% pretreated) was reported.

Rituximab in combination with other chemotherapeutic agents

The results of the studies are summarized in Table 4.

Rituximab plus bendamustine

Recently the particular mechanism of action of bendamustine, an alkylating agent with additional properties of a purine analog, has reawakened interest in this drug that has been extensively studied in indolent NHL and in CLL.[91] The preclinical observations demonstrating synergistic pro-apoptotic effects of bendamustine and rituximab (BR) supported clinical studies combining the two agents.

The GCLLSG initiated the CLL2M phase II study to investigate the combination of bendamustine at 70 mg/m^2

Table 3 Rituximab in combination treatment with pentostatin or cladribine

Authors	Comp. study	No. evaluable pts	Disease status	Regimen	Response	Follow-up
Lamanna et al[84]	No	32	Pretreated	PCR: P 4 mg/m^2 iv d 1, CTX 600 mg/m^2 iv d 1, R 375 mg/m^2 iv d 1 omitted from cycle 1	24 (75%) OR 8 (25%) CR	Median DoR: 25 mo Median TTF: 40 mo, NR for CR
Kay et al[85]	No	64	Untreated	PCR: P 2 mg/m^2 iv d 1, CTX 600 mg/m^2 iv d 1, R 375 mg/m^2 iv d 1 every 21 d for 6 cycles	58 (91%) OR 26 (41%) CR	Median DoR: 34 mo Median PFS: 32.6 mo
Shanafelt et al[86]	No	46 < 70 y	Untreated	PCR: P 2 mg/m^2 iv d 1, CTX 600 mg/m^2 iv d 1, R 375 mg/m^2 iv d 1 for 6 cycles	43 (93%) OR 19 (41%) CR	PFS not different between the 2 age groups
		18 ≥ 70 y			15 (83%) OR 7 (39%) CR	
Reynolds et al[87]	Yes	184	80% untreated	PCR: P 4 mg/m^2 iv d 1, CTX 600 mg/m^2 iv d 1, R 375 mg/m^2 iv d 1 every 21 days	41 (45%) OR	No differences in survival detected
			20% pretreated	versus FCR: F 20 mg/m^2 iv d 1–5, CTX 600 mg/m^2 iv d 1, R 375 mg/m^2 iv d 1 every 28 days	6 (7%) CR 53 (57.5%) OR 15 (17%) CR	
Kay et al[88]	No	33	Untreated	PR: P 4 mg/m^2 iv d 1, R 100 mg iv d 1, 375 mg/m^2 iv d 3 and d 5 for cycle 1, 375 mg/m^2 iv d 1 cycles 2–6; cycles were administered every 21 days	25 (76%) OR 9 (27%) CR	Median DoR 10.8 mo Median TFS 15.8 mo Median TTR 13.6 mo
Robak et al[89]	No	18	Pretreated	RC: R 375 mg/m^2 iv d 1, 2-CdA 0.12 mg/kg/d iv d 2–6 every 28 days	12 (67%) OR 1 (6%) CR	Median PFS 12 mo
		28		RCC: R 375 mg/m^2 iv d 1, 2-CdA 0.12 mg/kg iv d 2–4, CTX 250 mg/m^2 iv d 2–4 every 28 days	22 (78%) OR 2 (7%) CR	
Bertazzoni et al[90]	No	42 28 CLL/14 SLL	62% untreated 38% pretreated	R 375 mg/m^2 iv d 1, 2-CdA 0.1 mg/kg/d sc d 2–6 every 28 days	37 (88%) OR 21 (50%) CR	

Abbreviations: comp., comparative; P, pentostatin; R, rituximab; CTX, cyclophosphamide; 2-CdA, cladribine; pts, patients; d, days; OR, overall response; CR, complete response; DoR, duration of response; TTF, time to treatment failure; PFS, progression-free survival, TTR, time to retreatment; TFS, time-free survival; NR, not reached; mo, months; SLL, small lymphocytic lymphoma; iv, intravenous; sc, subcutaneous.

Table 4 Chemotherapeutic regimens including rituximab

References	Comp. study	No. evaluable pts	Prior therapy	Treatment regimen	Clinical response		Survival/duration of response
					CR (%)	OR (%)	
Fischer et al[92]	No	62	Pretreated	BR: B 70 mg/m^2 iv d 1–2, R 500 mg/m^2 iv d 1 (375 mg/m^2 iv first cycle)	15	77	Not evaluable
Fischer et al[93]	No	117	Untreated	BR: B 90 mg/m^2 iv d 1–2, R 500 mg/m^2 iv d 1 (375 mg/m^2 iv first course), every 4 weeks for up to 6 cycles every 4 weeks	33	91	Median PFS not reached after 18 mo of follow-up
Weide et al[94]	No	39	Pretreated	BMR: B 90 mg/m^2 iv d 2, 8 every 4 weeks R 375 mg/m^2 iv d 8, 15, 22, 29	10	92	Median TTR: 13 mo
Hillmen et al[95]	No	47	Untreated	R 375 mg/m^2 iv d 1 cycle 1, 500 mg/m^2 iv cycles 2–6, Chl d 1–7 10 mg/m^2/d oral every 28 days for 6 cycles; a further 6 cycles of Chl alone permitted in pts with continuing clinical response at 6 cycles	–	84	Not evaluable
Hillmen et al[98]	Yes	46	Pretreated	FCM: F 24 mg/m^2 oral d 1–5, CTX 150 mg/m^2 oral d 1–5, MIT 6 mg/m^2 iv d 1 for up to 6 courses	16	57	Not evaluable
				FCM-R: F 24 mg/m^2 os d 1–5, CTX 150 mg/m^2 oral d 1–5, MIT 6 mg/m^2 d 1, R 500 mg/m^2 d 1 (375 mg/m^2 first course) for up to 6 courses every 4 weeks	43	70	
Bosch et al[99]	No	72	Untreated	FCM-R: F 25 mg/m^2 iv d 1–4, CTX 250 mg/m^2 d 1–4, MIT 6 mg/m^2 d 1, R 375–500 mg/m^2 iv d 1 every 4 weeks	82	93	Not evaluable
Faderl et al[100]	No	30	Untreated	FCM-R: F 25 mg/m^2 iv d 2–4 cycle 1, d 1–3 cycles 2–6, CTX 250 mg/m^2 iv d 2–4 cycle 1, d 1–3 cycles 2–6, MIT 6 mg/m^2 iv d 2 cycle 1, d 1 cycles 2–6, R 500 mg/m^2 iv d 1 (375 mg/m^2 iv first cycle) every 4 weeks	83	96	After a median follow-up of 38.5 mo: TTF NR
Tsimberidou et al[101]	No	30	Pretreated	OFAR (oxaliplatin dose finding): O 17.5, 20, or 25 mg/mg/m^2 iv d 1–4, F 30 mg/m^2 iv d 2–3, cytarabine 1 g/m^2 iv d 2–3, R 375 mg/m^2 iv d 1 or d 3 every 4 weeks for up to 6 courses	7	33	Median DoR 10 mo At 6 mo: FFS 48% and OS 89%
Tsimberidou et al[104]	No	67	Pretreated	OFAR2: O 30 mg/m^2 iv d 1–4, R 375 mg/m^2 iv d 3, and pelfigrastim 6 mg d 6, F 30 mg/m^2 iv and cytarabine 500 mg/m^2 iv d 2–3 (dose level 1) d 2–4 (dose level 2) or d 2–5 (dose level 3) every 4 weeks	4	63	Median survival duration 21 mo

Abbreviations: comp., comparative; B, bendamustine; R, rituximab; CTX, cyclophosphamide; F, fludarabine; O, oxaliplatin; MIT, mitoxantrone; Chl, chlorambucil; pts, patients; d, days; OR, overall response; CR, complete response; DoR, duration of response; TTF, time to treatment failure; PFS, progression-free survival; NR, not reached; mo, months; iv, intravenous; sc, subcutaneous.

on two consecutive days plus rituximab on day 1 at a dose of 375 mg/m^2 for the first course and 500 mg/m^2 during subsequent cycles in 81 relapsed CLL patients.[92] BR treatment was administered every 28 days for up to six courses. In the 62 patients assessable for response, the ORR was 77.4% with a CR rate of 14.5% of patients. Of note is that a high ORR (92%) was observed among patients with del11q. Major but tolerable treatment toxicities were myelosuppression and infections. Grade 3–4 infections were documented in 5% of all given cycles.

Based on these interesting results BR was considered in first-line treatment in a multicenter Phase II study of the GCLLSG.[93] Preliminary results on 117 CLL patients showed an ORR of 90.9% with a CR in 32.7% of cases. After 18 months 75.8% of the patients were still in remission with a median PFS not reached. Grade 3–4 infections occurred in 5% of all administered courses. As in the previously treated patients, BR confirmed its efficacy even among patients with adverse prognostic features such as del11q and unmutated IgVH status, leading to ORR of 90.5% and 88.9%, respectively. Patients with del17p achieved only PRs (42.9%). The GCLLSG is presently investigating the efficacy of BR in comparison with fludarabine-based immunochemotherapy (FCR) as first-line treatment of CLL within a randomized Phase III trial (CLL10 protocol).

In a recently published retrospective trial, Weide et al evaluated 39 elderly patients with relapsed or refractory CLL who were treated with bendamustine, mitoxantrone and rituximab (BMR).[94] Treatment consisted of bendamustine 90 mg/m^2 on days 1 to 2, mitoxantrone 6 mg/m^2 on day 1 and rituximab 375 mg/m^2 on days 8, 15, 22 and 29. ORR was 92% (10% CR, 82% PR), with many patients receiving only a single cycle of immunochemotherapy. Median time to next CLL therapy was 13 months (0–69). Therapy was well tolerated, with two observed therapy-associated hospitalizations. A reversible grade 3–4 hematological toxicity was seen in 30 patients (77%).

Rituximab plus chlorambucil

Despite the increasing use of fludarabine-containing regimens and more recently FCR, chlorambucil remains a first-line treatment option, particularly for elderly patients and those with co-morbidities.[16] However, rates of CR obtained with the alkylating agents are relatively low (up to 7%) as are overall responses (approximately 65%). In the United Kingdom (UK) a phase II study is ongoing designed with the aim of assessing the feasibility of adding rituximab to chlorambucil in order to improve outcomes.[95] Treatment consisted of rituximab (375 mg/m^2 day 1 in the first cycle, 500 mg/m^2 in cycles 2–6) plus chlorambucil (10 mg/m^2 days 1–7) repeated every 28 days for six cycles. A further six cycles of chlorambucil alone was permitted in patients with continuing clinical response at six cycles. The interim analysis on 50 patients showed an ORR of 84%, which is 17.3% higher than in the well-matched subset of patients from the UK LRF CLL4 study treated with chlorambucil in monotherapy.[20] The high median age of patients entered in the study and the good tolerance, even in older patients, suggest that this combination treatment may be considered in those patients who cannot tolerate a more intensive regimen.

Rituximab plus fludarabine, cyclophosphamide, and mitoxantrone

The combination fludarabine, cyclophosphamide, and mitoxantrone (FCM) has been extensively, used demonstrating a high effectiveness in CLL patients both previously untreated and treated.[21,96,97] The next logical development of this treatment program was to investigate the role of rituximab addition (FCM-R). Hillmen et al reported the results of a small Phase II randomized trial which attempted to compare FCM with FCM-R in pretreated patients. CR rate was higher in patients receiving the monoclonal antibody (43%) even though the small number of patients did not allow statistically valid conclusions. Once again, this trial demonstrated that the addition of the monoclonal antibody did not increase toxicity, as the rate of serious adverse events was similar in the two treatment arms.[98]

Bosch et al treated 72 naïve CLL patients with this combination, obtaining a 93% ORR including 82% CRs, of which 46% were MRD negative, using multiparametric flow-cytometry. Results of the FCM clinical study were retrospectively compared with those of the immunochemotherapeutic regimen. Although the ORR rate was similar with the two treatment programs, the proportions of CRs and MRD negativity were significantly higher in patients receiving rituximab.[99] Variables associated with a lower CR, even with immunochemotherapy, included beta2-microglobulin and del17p. Severe neutropenia and infections were higher in patients treated with FCM-R even when comparing only the group of younger patients (age <65 years).

The same regimen with a higher dosage of rituximab (FCM-R) was investigated by Faderl et al in a pilot study of 30 untreated CLL patients in an attempt not to compare immunochemotherapy with chemotherapy, but to evaluate whether mitoxantrone addition to FCR provided any substantial benefit.[100] The authors concluded that compared to

FCR the addition of mitoxantrone does not seem to give more benefit when looking at clinical flow-cytometry or molecular responses. Further follow-up is required to assess the benefit in terms of time to treatment failure. The frequency of severe neutropenia was similar between FCM-R and FCR and in general the addition of mitoxantrone was associated with a greater myelosuppressive effect, since every patient received hematopoietic growth factor. The small number of patients enrolled in this study does not allow conclusions to be drawn on the influence of mitoxantrone on particular subgroups of patients.

Rituximab plus fludarabine, oxaliplatin, and cytarabine

Rituximab was also combined with fludarabine, oxaliplatin, and cytarabine (OFAR) in a Phase I/II dose-finding study for patients with fludarabine-refractory CLL.[101] After a median number of two courses, ORR achieved in this high-risk group of patients was 33%, with responses observed only in cases treated with the higher oxaliplatin dosage of 25 mg/m^2. Responses were high, 33%, even in the subset of patients carrying the 17p deletion. The superiority of OFAR compared with other cisplatin-based therapies could be attributed to the substitution of oxaliplatin for cisplatin, the considerable dose of cytarabine administered and the addition of the monoclonal anti-CD20 antibody.[102,103] A subsequent study (OFAR2) with rituximab in association with the same chemotherapeutic agents, administered at different dose levels (higher oxaliplatin, lower cytarabine dosages), confirmed the high efficacy of the combination in 52 refractory CLL patients. A response was achieved in 63% of patients, with a high antileukemic activity in patients with del17p; median survival duration was 21 months.[104]

Rituximab in combination with monoclonal antibodies

The rationale of combination treatment with monoclonal antibodies is based on: suboptimal efficacy of single-agent monoclonal antibody therapy, different molecular targets of the antibodies and different mechanism of action.

Rituximab and alemtuzumab

Alemtuzumab is a monoclonal humanized antibody specific for the CD52 antigen which is expressed at high levels in CLL cells and is active in initial and salvage therapy.[105] Treatment with alemtuzumab is warranted in patients with del17p and/or p53 mutations, both of which have been associated with resistance to most other available CLL treatment agents.

A pilot study to evaluate efficacy and tolerability of the combination of rituximab and alemtuzumab was performed in 12 heavily pretreated patients failing purine analogs therapy. Patients were treated in three different cohorts: rituximab 375 mg/m^2 was administered weekly for 4 weeks while alemtuzumab dosage was escalated in the different cohorts according to toxicity (3 mg, 10 mg, 30 mg, respectively, 3 times a week for 4 weeks).[106] Although only one patient achieved an objective response, the combination of the two monoclonal antibodies was shown to be safe as no significant myelosuppression was noted and none of the patients developed an opportunistic infection. Cytomegalovirus (CMV) reactivation was not detected in this trial but it should be emphasized that CMV antigenemia was not routinely performed. In an MD Anderson Cancer Center trial the administration with rituximab of the higher dosage of alemtuzumab, 30 mg iv 3 times a week, led to a higher response rate with at least one episode of infection in 52% of treated patients,[107] although none of the infections was fatal. Because a wide range of diagnoses was included in this series, it is difficult to draw any firm conclusion on whether the combination of rituximab and alemtuzumab has any advantage over single-agent monoclonal antibody therapy. In a more recent study, on only CLL patients, the same authors reported on the efficacy of the combination of the two monoclonal antibodies administered at a higher dosages with a different schedule.[108] ORR (53%) and time to treatment failure were similar to those in the previous study, with a trend toward a higher CR rate (18%). Documented infections occurred in 28% of patients but even in this study no fatal infections or opportunistic infections were recorded.

The interest in this combination of two monoclonal antibodies led to its efficacy being investigated as front-line treatment. Frankfurt et al reported the results of subcutaneous alemtuzumab administration in 20 previously untreated and symptomatic patients.[109] A high CR rate was achieved (40%), including all patients with del11q abnormality. Furthermore at the completion of therapy 70% of patients had no evidence of MRD by flow-cytometry.

Zent et al used the combination in untreated asymptomatic patients who had at least one marker of high-risk disease: del17p, del11q or combination of unmutated IgVH and CD38+/ZAP-70+.[110]

In both these first-line treatment studies, patients with CMV reactivation were described (30% and 10%, respectively). A low incidence of infections was reported: one patient in the first study developed CMV disease with full recovery after foscarnet, one patient in the second study was hospitalized after neutropenic fever.

Although the combination of the two monoclonal antibodies was demonstrated to be effective, with a low rate of severe infections, the profound immunosuppression documented warrants further investigation in particular subsets of patients, providing careful monitoring of complications.

One possible way to further increase the efficacy observed with FCR might be the addition of the combination of another monoclonal antibody targeting a different antigen.

At the MD Anderson Cancer Center, alemtuzumab 30 mg iv on days 1, 3, and 5 was added to FCR (CFAR regimen) in a first Phase II study with 79 refractory patients. An ORR of 65% and a CR rate of 24% were observed, patients who obtained CR showed a negative by flow-cytometry MRD.[111] As for most salvage treatments CFAR was more active in patients who were sensitive to their previous fludarabine regimen (ORR 74%, CR 36%) than in fludarabine-refractory patients (ORR 49%, CR 6%). It should be emphasized that the regimen was shown to be effective in 44% of patients presenting with 17p deletion. The incidences of major infections (11%), minor infections (28%) and fever of unknown origin (36%) were similar to those observed with FCR in the relapsed setting.

Given these promising results, this combination was subsequently administered as first-line treatment in patients with high-risk features determined as either 17p deletion and/or a high level of beta2-microglobulin (twice the upper normal level).[112] ORR achieved in the whole population was 92%, with 70% of CRs. A CR of 57% was observed in patients with del17p, with an ORR of 78%. Results and toxicities were compared

The addition of alemtuzumab conferred a higher CR rate and bone marrow MRD negativity in the same group of high-risk patients who received FCR only. After the CFAR regimen a higher rate of myelosuppression and CMV reactivation were observed. Incidence of other infections was similar. Treatment discontinuation was observed more often in patients receiving CFAR. The short follow-up of 24 months did not reveal differences in time to progression and OS between CFAR and the historical FCR control group. Longer follow-up will help to determine the role of CFAR in high-risk patients, although the response rate observed in these patients is the highest reported to date.

Rituximab and lumiliximab

Lumiliximab, a monoclonal antibody that binds specifically to CD23, has been tested in association with FCR in 31 refractory/relapsed patients.[113] Although the OR the results observed in this study are very similar to the others reported with FCR alone, the addition of lumiliximab increased CR twofold (65%). Importantly, the addition of the second monoclonal antibody did not increase toxicity. A large global randomized study comparing FCR with FCR plus lumiliximab in previously treated patients is ongoing.

Rituximab in combination with nonchemotherapeutic agents

Rituximab plus steroids

High-dose methylprednisolone (HDMP) is an established treatment in refractory CLL and can induce responses both in bulky disease and p53 mutation.[114,115] In preclinical studies rituximab was show to act synergistically with methylprednisolone to induce apoptosis of CLL cells, particularly in the presence of nurse-like cells.

Castro et al were the first to describe the combination of HDMP 1 g/m^2 for 5 days with rituximab in refractory patients, reporting excellent response rates (93%) and tolerability.[116] Similar results, summarized in Table 5, were observed in the Mayo Clinic retrospective study and in smaller series of heavily pretreated patients (ORR 93% to 75%, median PFS or duration of response 7 to 14 months).[117–119] In all series a high activity of the combination was reported in patients with aggressive disease features including those with del17p and/or bulky adenopathy, although in all studies a significantly high rate of infection (21.4% to 29%) was observed.

Castro et al conducted a study to evaluate dosing and toxicity of the HDMP and rituximab combination in chemotherapy-naïve patients.[120] Patients received HDMP 1 g/m^2 for 3 days; rituximab was administered in the first group at the dosage of 375 mg/m^2 for 12 doses and in the second group at a higher dosage of 750 mg/m^2 for 9 doses. ORR was 96% with 32% of CRs, 8% of which were MRD negative. It has to be emphasized that all patients >70 years responded to treatment and all cases with del11q and del17p achieved an objective response. No statistical difference in response was observed when patients were categorized according to the rituximab dosage. Median PFS and treatment-free survival were 30.5 and 33.3 months, respectively, and for CR patients median PFS was 40.3 months.

Rituximab and granulocyte-macrophage colony-stimulating factor

Although the synergistic effect of rituximab with chemotherapeutic agents is well known the toxicity of immunochemotherapeutic regimens remains a limitation. The availability of agents that may enhance rituximab's efficacy without the

Table 5 Rituximab in combination with high-dose steroids

Authors	No. evaluable pts	Disease status	Regimen	Response	Follow-up
Castro et al[116]	14	Pretreated	HDMP 1 g/m²/d iv for 5 days plus rituximab 375 mg/m² iv d 1, 8, 15, 22 every 4 weeks	13 (93%) OR 5 (36%) CR	Median TTP 15 m Median TFS: 22 m
Quinn et al[117]	12	Pretreated	Rituximab (dose not stated) plus HDMP 1 g/m²/d iv for 5 days or HDD 40 mg/d iv for 4 days every 4 weeks	9 (75%) OR 1 (8%) CR	Median DoR: 14 m
Bowen et al[118]	37	Pretreated	HDMP 1 g/m²/d iv for 5 days plus rituximab 375 mg/m² iv d 1, 8, 15, 22 every 4 weeks	29 (78%) OR 8 (22%) CR	Median PFS: 1 y 3 y survival: 41,3%
Dungarwalla et al[119]	14	Pretreated	HDMP 1 g/m²/d iv for 5 days plus rituximab 375 mg/m² iv d 1 every 28 days	13 (93%) OR 2 (14%) CR	Median PFS: 7 m Median OS: 20 m
Castro et al[120]	28	Untreated	HDMP 1 g/m² iv d 1–3 for 3 courses plus Group 1: rituximab 375 mg/m² iv 12 doses Group 2: rituximab 750 mg/m² iv 9 doses	27 (96%) OR 9 (32%) CR	Median FU 3 y: OS 96% Median PFS 30.5 m Median TFS 33.3 m

Abbreviations: pts, patients; HDMP, high-dose methylprednisolone; HDD, high-dose dexamethasone; iv, intravenous; d, days; y, year; OR, overall response; CR, complete response; TTP, time to progression; PFS, progression-free survival; TFS, treatment-free survival; DoR, duration of response; FU, follow-up.

myelosuppressive effects of chemotherapy has led to the investigation of cytokine partners.

As the lower density of CD20 antigen in CLL is considered one of the most important factors responsible for reduced rituximab activity, one approach to enhance responses could be the use of cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin-4, to upregulate CD20.[121]

The rationale for using GM-CSF in combination with rituximab is based on the observation of Venugopal et al that CLL patients exposed in vitro to GM-CSF have increased expression of surface CD20, potentially making them a better target for rituximab.[121] Furthermore the addition of GM-CSF in vitro enhances rituximab-induced ADCC against CLL cells, stimulating granulocyte and macrophage natural cytotoxicity.[122]

A phase II study conducted at the MD Anderson Cancer Center demonstrated the efficacy of the combination of rituximab at the standard dose of 375 mg/m² weekly for 4 weeks combined with GM-CSF 250 μg subcutaneously 3 times a week for 8 weeks.[123] The ORR observed in the 118 patients treated was 65% with 9% of CRs, 10% of nodular PRs and 53% PRs. The results suggested an improvement in response rate with the combination treatment over rituximab in monotherapy either in untreated or recurrent disease patients. The most common toxicity observed was mild erythema at the site of GM-CSF injection; six episodes of major infections were documented.

Considering the favorable results obtained with GM-CSF plus rituximab and considering the severe myelosuppressive effects observed after immunochemotherapy regimens, GM-CSF could play a role as a myeloid growth factor and in addition synergize with rituximab.

Ferrajoli et al recently reported the preliminary data of GM-CSF given in combination with FCR regimen in frontline treatment.[124] The study confirmed the achievement of a high ORR (100%) and CR (72%) rates. Of note, 85% of patients treated with this combination completed all 6 planned courses and only two patients (4%) received fewer than four courses.

Rituximab plus lenalidomide

Lenalidomide is an immunomodulatory drug that is a more potent analog of thalidomide. In patients with relapsed/refractory CLL, treatment with single-agent lenalidomide induced an ORR of 32% to 47% when used as monotherapy.[125,126] The combination of rituximab and lenalidomide was investigated at the MD Anderson Cancer Center based on the possible enhanced activity of rituximab induced by lenalidomide which stimulates natural killer (NK) cell proliferation.[127]

Patients were treated with rituximab (375 mg/m²) iv on days 1, 8, 15 and 22 of cycle 1, and then once every 4

weeks during cycles 3 to 12. Lenalidomide was given orally at the dose of 10 mg/day starting on day 9 of cycle 1 and continued daily for 12 cycles. Recently the results on 37 refractory relapsed patients treated with at least six courses have been reported.[128] The ORR (68%) was superior to that with single-agent lenalidomide despite all patients having received prior rituximab. Furthermore no differences in responses among the different prognostic groups were observed. Interestingly, the percentage of $CD19^+CD20^+$ B-cells decreased, and the percentages of $CD4^+$ T, $CD8^+$ T, $CD4^+CD25^{hi}CD127^-$ regulatory T and $CD3^-CD16^+CD56^+$ NK cells significantly increased.

Rituximab in maintenance therapy

Rituximab is an attractive candidate for maintenance therapy due to a favorable combined efficacy and safety profile, thus allowing extended therapy to improve outcome without compromising quality of life. In low-grade NHL, rituximab maintenance treatment clearly suggests an improvement in outcome following responses obtained with initial rituximab monotherapy or either chemotherapy or immunochemotherapy.[129–131]

Hainsworth et al administered consolidation treatment with rituximab in four weekly courses, at 6-month intervals, for 2 years in those CLL patients responding or showing a stable disease after initial front-line rituximab monotherapy.[59] Consolidation treatment increased ORR from 51% to 58%, and CR rate from 4% to 9%. The median PFS at the time of reporting was 18.6 months with a projected 1-year and 2-year PFS of 62% and 49%, respectively. Long-term toxicity during rituximab maintenance therapy was reported to be mild.

In a similar study rituximab consolidation was evaluated in 28 patients in CR or PR with positive flow-cytometric MRD after fludarabine treatment.[132] Rituximab was initially administered for four weekly doses, then MRD-positive patients received 4 monthly cycles of rituximab at 375 mg/m^2 followed by 12 monthly doses at 150 mg/m^2. Postinduction therapy significantly increased response duration compared with controls (87% vs 32% at 5 years). Updated results of this study, recently reported, confirmed the long PFS of consolidated patients from the end of induction treatment (40% at 9 years).[133]

A response-adjusted and flexible low-dose rituximab maintenance regimen was designed for relapsed CLL patients who achieved a partial or minimal response to prior therapy with rituximab.[134] Patients received the monoclonal antibody at the dosage of 100 mg on a 4-week schedule, if disease progression occurred during the interval between treatments was reduced or 500 mg of rituximab was given every 2 to 4 weeks. Maintenance therapy was successfully carried out in ≥6 months in 7 of the 12 enrolled patients with mild long-term toxicity. In three cases partial remission or minor response was maintained for relatively long periods (up to 42 months).

Conclusion

In the past 20 years considerable progress had been made in molecular and cellular biology, and prognosis and treatment of patients with CLL. These achievements have provided the basis for the development of innovative and more effective therapies in this disease. Two reports[135,136] recently published demonstrated, by comparing cohorts of patients from the 1980s and the 2000s, that survival in CLL patients had significantly improved. These data suggest that improved therapies, particularly in younger patients, might improve OS.

The introduction of rituximab has revolutionized the treatment of lymphoproliferative disorders. In CLL rituximab monotherapy has limited activity in refractory/relapsed patients, with responses being generally inferior to those seen in NHL. Although increasing dose intensity and frequency leads to higher response rates, even in first-line treatment, responses are almost always partial and of short duration. Furthermore the high dosages used are not feasible in routine clinical practice.

On the basis of preclinical evidence of the synergism between rituximab and fludarabine, rituximab has been incorporated into fludarabine-based regimens. Concurrent fludarabine and rituximab has been shown to increase ORR, CR and PFS but not long-term survival benefit. A further amelioration of the quality of responses has been achieved with the FCR. The improvement in PFS resulted directly from an improved ability to eradicate MRD, highlighting the importance of MRD eradication. The CLL8 German randomized trial is the first study demonstrating the superiority of the FCR arm over the comparator (FC) arm in prolonging OS. Based on the demonstrated benefit of FCR treatment, the US Food and Drug Administration has recently approved the new indication of this anti-CD20 monoclonal antibody as treatment of naïve or relapsed CLL, in combination with standard cytotoxic chemotherapy consisting of fludarabine and cyclophosphamide. Despite the demonstrated efficacy of FCR, important issues must still be addressed. Should FCR be considered the 'standard' care for all patients?

Selection of the most appropriate initial therapy in CLL must be based primarily on patient characteristics such as age, performance status, co-morbidities and biological disease characteristics. Although the addition of rituximab to FC improved outcome in patients with p53 mutation or del17p, results with this combination treatment are still unsatisfactory so that alternative treatments such as alemtuzumab or investigational therapies should be considered. As of now, FCR should be considered the standard care in young, fit patients in which the reasonable goal of treatment is CR, possibly MRD-negative CR. Although FCR toxicity is manageable and infections are similar to those observed after chemotherapy alone, the high rate of profound myelosuppression, persisting in some cases after the end of therapy, warrants adequate monitoring prophylaxis and high standard of care.

Management decisions can be different in elderly patients with comorbidities, as FCR may be poorly tolerated. This group of patients may benefit from other combination regimens including rituximab (eg, PCR, BR), or alternatively first-line alemtuzumab treatment if severe CLL-related pancytopenia is present.

A large randomized multicenter study showed that results obtained after FCR treatment in clinical trials are not reproducible in the community setting where the majority of patients are treated. Furthermore what has not been answered in a randomized fashion is whether FCR is superior, with a similar tolerability, to other combination regimens such as PCR, FCR-Lite, BR, and R-FCM.

Even if high CR rates with eradication of MRD have been obtained with FCR, a proportion of patients still show a poor outcome. To overcome resistance in the high-risk group of patients new treatment strategies are under investigation. Preliminary results have shown that the FCR regimen with the addition of alemtuzumab is effective in treating high-risk patients in first-line treatment. Opportunistic infections related to concomitant depletion of T- and B-cell lymphocytes when alemtuzumab and rituximab are administered should be strictly monitored. Longer follow-up of the study is needed in order to assess if an advantage in PFS and/or OS is recorded in poor-risk CLL.

Different strategies with nonchemotherapeutic agents are under investigation. The combination of rituximab and lenalidomide has been shown to be more effective than lenalidomide alone in relapsed/refractory patients, even in cases showing adverse prognostic features and bulky disease.

Additional studies based on biological stratification with an adequate follow-up are warranted in order to assess the impact of rituximab combined with new compounds.

Disclosure

The authors report no conflicts of interest.

References

1. National Cancer Institute SEER Cancer Statistics Review 1975–2007. http://seer.cancer.gov/csr/1975_2007/. Accessed 2010 Oct 30.
2. Dighiero G, Hamblin TJ. Chronic lymphocytic leukaemia. *Lancet.* 2008;371:1017–1029.
3. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*. 1981;48:198–206.
4. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. *Blood.* 1975;46:219–234.
5. Montillo M, Hamblin T, Hallek M, et al. Chronic lymphocytic leukemia: novel prognostic factors and their relevance for risk-adapted therapeutic strategies. *Haematologica*. 2005;90:391–399.
6. Montserrat E. Classical and new prognostic factors in chronic lymphocytic leukemia: where to now? *Hematol J*. 2002;3:7–9.
7. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343:1910–1916.
8. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood.* 1999;94:1840–1847.
9. Hamblin T, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94:1848–1854.
10. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*. 2003;348:1764–1775.
11. di Giovanni S, Valentini G, Carducci P, et al. Beta-2-microglobulin is a reliable tumor marker in chronic lymphocytic leukemia. *Acta Haematol*. 1989;81:181–185.
12. CLL Trialists' Collaborative Group. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. *J Natl Cancer Inst*. 1999;91:861–868.
13. Cheson BD, Bennet JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*. 1996;87:4990–4997.
14. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood*. 2008;111:5446–5456.
15. Keating MJ, O'Brien S, Kantariian, et al. Long-term follow-up of patients with chronic lymphocytic leukemia treated with fludarabine as a single agent. *Blood.* 1993;81:2878–2884.
16. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med*. 2000;343:1750–1757.
17. Johnson S, Smith AG, Loffler H, et al. Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukaemia. The French Cooperative Group on CLL. *Lancet.* 1996;347:1432–1438.
18. Leporrier M, Chevret S, Cazin B, et al. Randomized comparison of fludarabine, CAP, and ChOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. *Blood*. 2001;98:2319–2325.

19. Keating MJ, O'Brien S, Lerner S, et al. Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. *Blood.* 1998;92:1165–1171.
20. Catovsky D, Richards S, Matutes E, et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. *Lancet*. 2007; 370:230–239.
21. Bosch F, Ferrer A, Lopez-Guillermo A, et al. Fludarabine, cyclophosphamide and mitoxantrone in the treatment of resistant or relapsed chronic lymphocytic leukaemia. *Br J Haematol*. 2002;119:976–984.
22. Steurer M, Pall G, Richards S, et al. Single-agent purine analogues for the treatment of chronic lymphocytic leukaemia: a systematic review and meta-analysis. *Cancer Treat Rev*. 2006;32:377–389.
23. Keating MJ, Coutrè S, Rai K, et al. Management guidelines for use of alemtuzumab in B-cell chronic lymphocytic leukemia. *Clin Lymphoma*. 2004;4:220–227.
24. Montserrat E. Rituximab in chronic lymphocytic leukemia. *Semin Oncol*. 2003;30:34–39.
25. Alas S, Emmanouilides C, Bonavida B. Inhibition of interleukin 10 by rituximab results in down-regulation of bcl-2 and sensitization of B-cell non-Hodgkin's lymphoma to apoptosis. *Clin Cancer Res*. 2001;7: 709–723.
26. di Gaetano N, Xiao Y, Erba E, et al. Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line resistant ot the cytotoxic activity of either drug alone. *Br J Haematol*. 2001;114: 800–809.
27. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood.* 2008;112:975–980.
28. Robak T, Dmoszynska A, Solal-Celigny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. *J Clin Oncol*. 2010;28:1756–1765.
29. Hallek M. State-of-the-art treatment of chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program.* 2009;440–449.
30. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol*. 2005; 23:4079–4088.
31. Hallek M, Fingerle-Rowson G, Fink AM, et al. First-line treatment with fludarabine (f), cyclophosphamide (C), and rituximab (R) (FCR) improves overall survival (OS) in previously untreated patients (pts) with advanced chronic lymphocytic leukemia (CLL): results of a randomized phase III trial on behalf of an international group of investigators and the German CLL Study Group. *Blood.* 2009;114:Abstract 535.
32. Moreton P, Kennedy B, Lucas G, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. *J Clin Oncol*. 2005;23: 2971–2979.
33. Dreger P, Corradini P, Kimby E, et al. Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. *Leukemia*. 2007;21:12–17.
34. Tobinai K. Rituximab and other emerging antibodies as molecular target-based therapy of lymphoma. *Int J Clin Oncol*. 2003;8:212–223.
35. Smith MR. Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene*. 2003;22:7359–7368.
36. Ginaldi L, de Martinis M, Matutes E, et al. Levels of expression of CD19 and CD20 in chronic B cell leukaemias. *J Clin Pathol*. 1998;51: 364–369.
37. Glennie MJ, French RR, Cragg MS, et al. Mechanisms of killing by anti-CD20 monoclonal antibodies. *Mol Immunol*. 2007;44:3823–3837.
38. Cragg MS, Walshe CA, Ivanoy AO, et al. The biology of CD20 and its potential as a target for mAb therapy. *Curr Dir Autoimmun*. 2005; 8:140–174.
39. Demidem A, Lam T, Alas S, et al. Chimeric anti-CD20 (IDEC-C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs. *Cancer Biother Radiopharm*. 1997;12:177–186.
40. Manches O, Lui G, Chaperot L, et al. In vitro mechanisms of action of rituximab on primary non-Hodgkin lymphomas. *Blood*. 2003;101: 949–954.
41. Harjunpaa A, Junnikkala S, Meri S. Rituximab (anti-CD20) therapy of B-cell lymphomas: direct complement killing is superior to cellular effector mechanisms. *Scand J Immunol*. 2000;51:634–641.
42. Cartron G, Dacheux L, Salles G, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood.* 2002;99:754–758.
43. Farag SS, Flinn IW, Modali R, et al. Fc gamma RIIIa and Fc gamma RIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia. *Blood.* 2004;103:1472–1474.
44. Treon SP, Mitsiades C, Mitsiades N, et al. Tumor cell expression of CD59 is associated with resistance to CD20 serotherapy in patients with B-cell malignancies. *J Immunother*. 2001;24:263–271.
45. Golay J, Zaffaroni L, Vaccari T, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. *Blood.* 2000;95: 3900–3908.
46. Golay J, Zaffaroni L, Vaccari T, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. *Blood*. 2000;95:3900–3908.
47. Pedersen IM, Kitada S, Schimmer A, et al. The triterpenoid CDDO induces apoptosis in refractory CLL B cells. *Blood*. 2002;100:2965–2972.
48. Selenko N, Majdic O, Jager U, et al. Cross-priming of cytotoxic T cells promoted by apoptosis-inducing tumor cell reactive antibodies? *J Clin Immunol.* 2002;22:124–130.
49. Selenko N, Majdic O, Draxier S, et al. CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross-priming of CD8+ cytotoxic T cells. *Leukemia.* 2001;15: 1619–1626.
50. Maloney DG, Grillo-Lopez AJ, Bodkin DJ, et al. IDEC-C2B8: Results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. *J Clin Oncol*. 1997;15:3266–3274.
51. Foran JM, Rohatiner AZ, Cunningham D, et al. European phase II study of Rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly diagnosed mantle-cell lymphoma, immunocytoma and small B-cell lymphocytic lymphoma. *J Clin Oncol*. 2000;18:317–324.
52. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. *J Clin Oncol*.1998;16:2825–2833.
53. Winkler U, Jensen M, Manzke O, et al. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (Rituximab, IDEC-C2B8). *Blood*. 1999;94:2217–2224.
54. Nguyen DT, Amess JA, Doughty H, et al. IDEC-C2B8 anti-CD20 (rituximab) immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoproliferative disorders: Evaluation of response on 48 patients. *Eur J Haematol*.1999;62:76–82.
55. Huhn D, von Schilling C, Wilhelm M, et al. Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. *Blood*. 2001;98:1326–1331.
56. Itala M, Geisler CH, Kimby E, et al. Standard-dose anti-CD20 antibody rituximab has efficacy in chronic lymphocytic leukaemia: results from a Nordic multicentre study. *Eur J Haematol*. 2002;69:129–134.
57. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J Clin Oncol*. 2001;19: 2165–2170.
58. Byrd JC, Murphy T, Howard RS, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. *J Clin Oncol*. 2001;19:2153–2164.
59. Hainsworth JD, Litchy S, Barton JH, et al. Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol*. 2003;21:1746–1751.

60. Thomas DA, O'Brien S, Giles FJ, et al. Single agent rituximab in early stage chronic lymphocytic leukemia. *Blood*. 2001;98:364a.
61. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med*. 2000;343:1750–1757.
62. Perz J, Topaly J, Fruehauf S, et al. Level of CD20 expression and efficacy of rituximab treatment in patients with resistant or relapsing B-cell prolymphocytic leukaemia and B-cell chronic lymphocytic leukaemia. *Leuk Lymphoma*. 2002;43:149–151.
63. Manshouri T. Circulating CD20 is detectable in the plasma of patients with chronic lymphocytic leukemia and is of prognostic significance. *Blood*. 2003;101:2507–2513.
64. Maloney DG, Grillo-Lopez AJ, Bodkin DJ, et al. IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. *J Clin Oncol*.1997;15:3266–3274.
65. Furlan A, Villanova F, Pietrogrande F, et al. Low-dose fludarabine increases rituximab cytotoxicity in B-CLL cells by triggering caspases activation in vitro. *Leuk Lymphoma*. 2010;51:107–113.
66. Chow KU, Sommerlad WD, Boehrer S, et al. Anti-CD20 antibody (IDEC-C2B8, rituximab) enhances efficacy of cytotoxic drugs on neoplastic lymphocytes in vitro: role of cytokines, complement, and caspases. *Haematologica*. 2002;87:33–43.
67. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). *Blood*. 2003;101:6–14.
68. Woyach JA, Ruppert AS, Heerema NA, et al. Treatment with fludarabine and rituximab produces extended overall survival (OS) and Progression-Free Survival (PFS) in chronic lymphocytic leukemia (CLL) without increased risk of second malignancy: long-term follow up of CALGB study 9712. *Blood*. 2009;114:Abstract 539.
69. Byrd JC, Rai K, Peterson BL, et al. Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. *Blood*. 2005;105:49–53.
70. Schulz H, Klein SK, Rehwald U, et al. Phase 2 study of a combined immunochemotherapy using rituximab and fludarabine in patients with chronic lymphocytic leukemia. *Blood*. 2002;100:3115–3120.
71. Hallek M, Schmitt B, Wilhelm M, et al. Fludarabine plus cyclophosphamide is an efficient treatment for advanced chronic lymphocytic leukaemia (CLL): results of a phase II study of the German CLL Study Group. *Br J Haematol.* 2001;114:342–348.
72. Flinn IW, Byrd JC, Morrison C, et al. Fludarabine and cyclophosphamide with filgrastim support in patients with previously untreated indolent lymphoid malignancies. *Blood*. 2000;96:71–75.
73. O'Brien SM, Kantarjian HM, Cortes J, et al. Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. *J Clin Oncol*. 2001;19:1414–1420.
74. Wierda W, O'Brien S, Wen S, et al. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2005;23:4070–4078.
75. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood.* 2002;99:3554–3561.
76. Rai KR, Freter CE, Mercier RJ, et al. Alemtuzumab in previously treated chronic lymphocytic leukemia patients who also had received fludarabine. *J Clin Oncol*. 2002;20:3891–3897.
77. Tsimberodou AM, Tam C, Abruzzo LV, et al. Chemoimmunotherapy may overcome the adverse prognostic significance of 11q deletion in previously untreated patients with chronic lymphocytic leukemia. *Cancer*. 2009;115:373–380.
78. Wierda W, O'Brien S, Faderl S, et al. A retrospective comparison of three sequential groups of patients with Recurrent/Refractory chronic lymphocytic leukemia treated with fludarabine-based regimens. *Cancer*. 2006;106:337–345.
79. Hallek M, Fingerle-Rowson G, Fink AM, et al. Immunochemotherapy with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) versus fludarabine and cyclophosphamide (FC) improves response rates and progression-free survival (PFS) of previously untreated patients (pts) with advanced chronic lymphocytic leukemia (CLL). *Blood*. 2008; 112:Abstract 325.
80. Lepretre S, Aurran T, Mahe B, et al. Immunochemotherapy with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) versus fludarabine (F), cyclophosphamide (C) and mabcampath (Cam) (FCCam) in previously untreated patients (pts) with advanced B-chronic lymphocytic leukemia (B-CLL): experience on safety and efficacy within a randomised multicenter phase III trial of the French Cooperative Group on CLL and WM (FCGCLL/MW) and the "Groupe Ouest-Est d'Etudes Des Leucémies Aigües Et Autres Maladies Du sang" (GOELAMS) :CLL2007FMP (for fit medically patients). *Blood*. 2009;114:Abstract 538.
81. Foon KA, Boyiadzis M, Land SR, et al. Chemoimmunotherapy with low-dose fludarabine and cyclophosphamide and high dose rituximab in previously untreated patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2009;27:498–503.
82. Lamanna N, Jurcic JG, Nov A, et al. Sequential therapy with fludarabine, high-dose cyclophosphamide, and rituximab in previously untreated patients with chronic lymphocytic leukemia produces high-quality responses: molecular remissions predict for durable complete responses. *J Clin Oncol*. 2009;27:491–497.
83. Weiss MA, Maslak PG, Jurcic JG, et al. Pentostatin and cyclophosphamide: an effective new regimen in previously treated patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2003;21: 1278–1284.
84. Lamanna N, Kalaycio M, Maslak P, et al. Pentostatin, cyclophosphamide, and rituximab is an active, well-tolerated regimen for patients with previously treated chronic lymphocytic leukemia. *J Clin Oncol*. 2006;24:1575–1581.
85. Kay NE, Geyer SM, Call TG, et al. Combination chemoimmunotherapy with pentostatin, cyclophosphamide, and rituximab shows significant clinical activity with low accompanying toxicity in previously untreated B chronic lymphocytic leukemia. *Blood.* 2007; 109:405–411.
86. Shanafelt TD, Lin T, Geyer SM, et al. Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia. *Cancer*. 2007;109:2291–2298.
87. Reynolds C, di Bella N, Lyons RM, et al. Phase III trial of fludarabine, cyclophosphamide, and rituximab vs pentostatin, cyclophosphamide, and rituximab in B-cell chronic lymphocytic leukemia. *Blood*. 2008; 112:Abstract 126.
88. Kay NE, Wu W, Kabat B, et al. Pentostatin and rituximab therapy for previously untreated patients with B-cell chronic lymphocytic leukemia. *Cancer*. 2010;116:2180–2187.
89. Robak T, Smolewski P, Cebula B, et al. Rituximab plus cladribine with or without cyclophosphamide in patients with relapsed or refractory chronic lymphocytic leukemia. *Eur J Haematol*. 2007;79: 107–113.
90. Bertazzoni P, Rabascio C, Gigli F, et al. Riruximab and subcutaneous cladribine in chronic lymphocytic leukemia for newly diagnosed and relapsed patients. *Leuk Lymphoma*. 2010;51:1485–1493.
91. Montillo M, Ricci F, Tedeschi A, et al. Bendamustine: new perspective for an old drug in lymphoproliferative disorders. *Expert Rev Hematol.* 2010;3:131–148.
92. Fischer K, Stilgenbauer S, Schweighofer CD, et al. Bendamustine in combination with rituximab (BR) for patients with relapsed chronic lymphocytic Leukemia (CLL): a multicentre phase II trial of the German CLL Study Group (GCLLSG). *Blood*. 2008;112: Abstract 330.
93. Fischer K, Cramer P, Stilgenbauer S, et al. Bendamustine combined with rituximab (BR) in first-line therapy of advanced CLL: A multicenter phase II trial of the German CLL Study Group (GCLLSG). *Blood*. 2009;114:Abstract 205.

94. Weide R, Hess G, Koppler H, et al. High anti-lymphoma activity of bendamustine/mitoxantrone/rituximab in rituximab pretreated relapsed or refractory indolent lymphomas and mantle cell lymphomas. A multicenter phase II study of the German Low Grade Lymphoma Study Group (GLSG). *Leuk Lymphoma*. 2007;48:1299–1306.
95. Hillmen P, Gribben JG, MD, Follows GA, et al. An open-label phase II study to investigate the safety and efficacy of rituximab plus chlorambucil in previously untreated patients with CD20-positive B-cell chronic lymphocytic leukaemia (CLL). *Blood*. 2009;114: Abstract 3428.
96. Bosch F, Ferrer A, Villamor N, et al. Fludarabine, cyclophosphamide, and mitoxantrone as initial therapy of chronic lymphocytic leukemia: high response rate and disease eradication. *Clin Cancer Res*. 2008; 14:155–1561.
97. Hendry L, Bowen A, Matutes E, et al. Fludarabine, cyclophosphamide and mitoxantrone in relapsed or refractory chronic lymphocytic leukemia and low grade non-Hodgkin's lymphoma. *Leuk Lymphoma*. 2004;45:945–950.
98. Hillmen P, Pocock C, Cohen D, et al. NCRI CLL201 Trial: a randomized phase ii trial of fludarabine, cyclophosphamide and mitoxantrone (FCM) with or without rituximab in previously treated CLL. *Blood*. 2007;110:Abstract 752.
99. Bosch F, Abrisqueta P, Villamor N, et al. Rituximab, fludarabine, cyclophosphamide, and mitoxantrone: a new, highly active chemoimmunotherapy regimen for chronic lymphocytic leukemia. *J Clin Oncol*. 2009;27:4578–4584.
100. Faderl S, Wierda W, O'Brien S, et al. Fludarabine, cyclophosphamide, mitoxantrone plus rituximab (FCM-R) in frontline CLL < 70 years. *Leuk Res*. 2010;34:284–288.
101. Tsimberidou AM, Wierda W, Plunkett W, et al. Phase I-II study of oxaliplatin, fludarabine, cytarabine, and rituximab combination therapy in patients with Richter's syndrome or fludarabine-refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2008;26(2):196–203.
102. Tsimberidou AM, O'Brien SM, Cortes JE, et al. Phase II study of fludarabine, cytarabine (Ara-C), cyclophosphamide, cisplatin and GM-CSF (FACPGM) in patients with Richter's syndrome or refractory lymphoproliferative disorders. *Leuk Lymphoma*. 2002;43: 767–772.
103. Giles FJ, O'Brien SM, Santini V, et al. Sequential cis-platinum and fludarabine with or without arabinosyl cytosine in patients failing prior fludarabine therapy for chronic lymphocytic leukemia: a phase II study. *Leuk Lymphoma.* 1999;36:57–65.
104. Tsimberidou AM, Wierda W, Plunkett W, et al. Oxaliplatin, fludarabine, cytarabine, and rituximab combination therapy induces high response rates in agressive chronic lymphocytic Leukemia (CLL) and Richter's syndrome (RS). *Blood*. 2009;114:Abstract 3443.
105. Rossmann ED, Lundin J, Lenkei R, et al. Variability in B-cell antigen expression: implications for the treatment of B-cell lymphomas and leukemias with monoclonal antibodies. *Hematol J*. 2001;2: 300–306.
106. Nabhan C, Patton D, Gordon LI, et al. A pilot trial of rituximab and alemtuzumab combination therapy in patients with relapsed and/or refractory chronic lymphocytic leukemia (CLL). *Leuk Lymphoma*. 2004;45:2269–2273.
107. Faderl S, Thomas DA, O'Brien S, et al. Experience with alemtuzumab plus rituximab in patients with relapsed and refractory lymphoid malignancies. *Blood*. 2003;101:3413–3415.
108. Faderl S, Ferrajoli A, Wierda W, et al. Alemtuzumab by continuous intravenous infusion followed by subcutaneous injection plus rituximab in the treatment of patients with chronic lymphocytic leukemia recurrence. *Cancer*. 2010;116:2360–2365.
109. Frankfurt O, Hamilton E, Duffey S, et al. Alemtuzumab and Rituximab Combination Therapy for Patients with Untreated CLL – a Phase II Trial. *Blood*. 2008;112:Abstract 2098.
110. Zent CS, Call TG, Shanafelt TD, et al. Early treatment of high-risk chronic lymphocytic leukemia with alemtuzumab and rituximab. *Cancer*. 2008;113:2110–2118.
111. Wierda WG, O'Brien S, Faderl S, et al. Combined cyclophosphamide, fludarabine, alemtuzumab, and rituximab (CFAR), an active regimen for heavily treated patients with CLL. *Blood*. 2006;108: Abstract 31.
112. Parikh SA, Keating M, O'Brien S, et al. Frontline Combined Chemoimmunotherapy with Fludarabine, Cyclophosphamide, Alemtuzumab and Rituximab (CFAR) in High-Risk Chronic Lymphocytic Leukemia. *Blood*. 2009;114:Abstract 208.
113. Byrd JC, Kipps TJ, Flinn IW, et al. Phase 1/2 study of lumiliximab combined with fludarabine, cyclophosphamide, and rituximab in patients with relapsed or refractory chronic lymphocytic leukaemia. *Blood*. 2010;115:489–495.
114. Thornton PD, Hamblin M, Treleaven JG, et al. High dose methyl prednisolone in refractory chronic lymphocytic leukaemia. *Leuk Lymphoma*. 1999;34:167–170.
115. Thornton PD, Matutes E, Bosanquet AG, et al. High dose methylprednisolone can induce remissions in CLL patients with p53 abnormalities. *Ann Hematol*. 2003;82:759–765.
116. Castro JE, Sandoval-Sus JD, Bole J, et al. Rituximab in combination with high-dose methylprednisolone for the treatment of fludarabine refractory high-risk chronic lymphocytic leukemia. *Leukemia.* 2008; 22:2048–2053.
117. Quinn JP, Mohamedbhai S, Chipperfield K, et al. Efficacy of rituximab in combination with steroids in refractory chronic lymphocytic leukemia. *Leuk Lymphoma*. 2008;49:1995–1998.
118. Bowen DA, Call TG, Jenkins GD, Zent CS, et al. Methylprednisolone-rituximab is an effective salvage therapy for patients with relapsed chronic lymphocytic leukemia including those with unfavorable cytogenetic features. *Leuk Lymphoma*. 2007;48: 2412–2417.
119. Dungarwalla M, Evans SO, Riley U, et al. High dose methylprednisolone and rituximab is an effective therapy in advanced refractory chronic lymphocytic leukemia resistant to fludarabine therapy. *Haematologica*. 2008;93:475–476.
120. Castro JE, Sandoval-Sus JD, Jain S, et al. Rituximab in combination with high-dose methylprednisolone for the treatment of chronic lymphocytic leukemia. *Leukemia*. 2009;23:1779–1789.
121. Venugopal P, Sivaraman S, Huang XK, et al. Effects of cytokines on CD20 antigen expression on tumor cells from patients with chronic lymphocytic leukemia. *Leuk Res*. 2000;24:411–415.
122. Voso MT, Pantel G, Rutella S, et al. Rituximab reduces the number of peripheral blood B-cells in vitro mainly by effector cell-mediated mechanisms. *Haematologica.* 2002;87:918–925.
123. Ferrajoli A. Incorporating the use of GM-CSF in the treatment of chronic lymphocytic leukemia. *Leuk Lymphoma*. 2009;50:514–516.
124. Ferrajoli A, Faderl S, O'Brien S, et al. Experience with fludarabine, cyclophosphamide, rituximab (FCR) plus GM-CSF in frontline therapy for chronic lymphocytic leukemia (CLL). *Blood (ASH Annual Meeting Abstracts).* 2008;112:Abstract 3173.
125. Chanan-Khan A, Miller KC, Musial L, et al. Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. *J Clin Oncol*. 2006;24: 5343–5349.
126. Ferrajoli A, Lee BN, Schlette EJ, et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood.* 2008;111:5291–5297.
127. Wu L, Adams M, Carter T, et al. Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. *Clin Cancer Res*. 2008;14: 4650–4657.
128. Ferrajoli A, Badoux XC, O'Brien S, et al. Combination Therapy with Lenalidomide and Rituximab in Patients with Relapsed Chronic Lymphocytic Leukemia (CLL). *Blood*. 2009;114:Abstract 206.
129. Ghielmini M, Schmitz SF, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly × 4 schedule. *Blood*. 2004;103:4416–4423.

130. Salles GA, Seymour JF, Feugier P, et al. Rituximab maintenance for 2 years in patients with untreated high tumor burden follicular lymphoma after response to immunochemotherapy. *J Clin Oncol.* 2010; 28:8004.
131. van Oers MHJ, Klasa R, Marcus RE, et al. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. *Blood*. 2006;108:3295–3301.
132. Del Poeta G, Del Principe MI, Buccisano F, et al. Consolidation and maintenance immunotherapy with rituximab improve clinical outcome in patients with B-cell chronic lymphocytic leukemia. *Cancer.* 2008; 112:119–128.
133. Del Poeta G, Del Principe MI, Ragusa D, et al. Rituximab maintenance following chemoimmunotherapy improves outcome in B-cell chronic lymphocytic leukemia. *Blood*. 2009;114:Abstract 2364.
134. Srock S, Schriever F, Neubauer A, et al. Long-term treatment with rituximab is feasible in selected patients with B-CLL: response-adjusted low-dose maintenance treatment with rituximab in patients with relapsed B-CLL, who achieved a partial or minimal response to prior rituximab therapy. *Leuk Lymphoma*. 2007;48:905–911.
135. Abrisqueta P, Pereira A, Rozman C, et al. Improving survival in patients with chronic lymphocytic leukemia (1980–2008): the Hospital Clinic of Barcelona experience. *Blood*, 2009;114:2044–2050.
136. Brenner H, Gonolons A, Pulte D. Trends in long-term survival of patients with chronic lymphocytic leukemia from the early 21st century. *Blood*, 2008;111:4916–4921.